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GROWTH RESPONSES OF BILOXI SOYBEANS TO VARIATION IN THE RELATIVE CONCENTRATIONS OF PHOSPHATE AND NITRATE IN THE NUTRIENT SOLUTION

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1940

By
CHARLES L. HAMNER

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GROWTH RESPONSES OF BILOXI SOYBEANS TO VARIATION IN RELATIVE CONCENTRATIONS OF PHOSPHATE AND NITRATE IN THE NUTRIENT SOLUTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 510

CHARLES L. HAMNER

(WITH FOUR FIGURES)

Introduction

Experimental results from many nutrient experiments have shown that, in general, if an element is lacking or low in concentration in the nutrient solution or soil solution, it will be lacking or present only at very low concentrations in the plant. Also, an essential element present in abundance in the nutrient solution will probably be present in abundance in the plant, although its concentration may vary with variation in environmental factors. A factor which may affect the accumulation of one element in the plant is the presence or absence of another essential element in the nutrient solution (2). The deficiency of an element or its abundance in the nutrient solution may affect the rate and amount of absorption of other elements, and thus affect growth responses.

It is probable that for every plant there is a specific nutrient requirement for maximum vegetative expression, flowering, fruit production, etc., and it is probable that this requirement changes as the conditions within the plant or external environments change. Just what constitutes optimum requirements will depend upon the viewpoint of the investigator, but many workers have utilized vegetative growth responses as a measure of the suitability of any particular nutrient solution. Two methods of measuring the growth responses of plants are possible, and may be classified as qualitative and quantitative. Quantitative measurements are concerned with the total accumulation of dry matter by the plant; qualitative measurements are concerned with the character or types of material composing the plant—proteins, fats, vitamins, etc. After a definite corre-

lation between quantitative response and nutrient requirements has been established, then an investigation of the qualitative responses may lead to an understanding or interpretation of the quantitative responses.

The object of the present study was a determination of the relationships existing between growth responses and the concentration of various ions in sand and solution culture. Since it is impossible to measure and record all the growth responses of any particular group of plants, and since the summation of the qualitative responses represent the quantitative responses, the criteria of response used here have been the accumulation of dry matter by the plant and its parts and the general appearance of the plant.

Experimental methods

Biloxi soybeans were used as experimental material. Several preliminary experiments were carried out, both in sand and solution culture, but the detailed results presented have been taken only from sand culture experiments, since plants grew as well in sand culture as in comparable solution cultures and the former experiments were more readily carried out. In all experiments, seeds were selected for uniformity and planted at an even depth in well-washed white quartz sand. They were then placed in darkness or in the greenhouse and watered with tap water. After germination, uniform seedlings were selected and transplanted to quartz sand contained in glazed 8×4-inch crocks with a $\frac{3}{8}$-inch hole in the bottom, each crock containing about 1500 cc. of sand. They were maintained on a well lighted greenhouse bench. After transplantation, the plants were irrigated with the various nutrients. The preliminary experiments were carried out in the early summer when the length of a natural day was in excess of the critical photoperiod (4). The final experiments were carried out in the late summer and the plants received supplementary illumination of an intensity of about 20 foot-candles from sunset to 2 A.M., so that the plants remained vegetative throughout the experimental period. No attempt was made to control greenhouse temperatures, although excessively high temperatures were avoided by careful ventilation. Excessively low atmospheric humidity was prevented by frequent spraying of the walks

and walls with water during warm dry days. The plants were watered each day with sufficient nutrient solution so that an excess dripped out of the openings in the bottom of the crocks; twice a week the pots were thoroughly flushed with distilled water, immediately followed by application of fresh nutrient solution.

Harvesting

In preliminary experiments, observations were made on the effect of an increase in the concentration of a single one of the major essential elements of the nutrient solution by adding its sodium salt or its chloride, depending upon whether the element under consideration was an anion or a cation. The procedure was to use a three salt solution (**8**), which promotes fairly active vegetative growth, but to use this at about one-fifth the concentration ordinarily employed. Growth of plants in this solution was compared with the growth obtained by adding various single salts to it.

The various nutrient solutions used and the results obtained are shown in table 1. The experiment was continued for 16 days after transplanting.

The result of the preliminary experiment showed that with a concentration one-fifth that of the complete nutrient solution used, or even with one-tenth concentration of the complete solution, active vegetative growth could be obtained without any deficiency symptoms appearing. The complete solution diluted to one-fifth concentration, to which KCl had been added, showed the greatest growth next to the complete solution. The complete solution diluted to one-fifth, to which NaH_2PO_4 had been added, showed evidence of toxicity. The complete solution diluted to one-fifth, to which NaCl had been added, also showed poor growth (table 1). It was not possible to tell whether the decreased growth was caused by Na or by Cl or both. It was also not possible to decide whether the toxicity was caused by Na or by the HPO_4.

Because of the difficulty of interpreting the results of the preliminary experiments, another method was utilized in making up the nutrient solutions. By this procedure two series of nutrient solutions were obtained. In one series the cations (Ca, Mg, and K) would vary in relation to one another while the anions (S, N, and P)

remained constant throughout the series. In the other, the anions varied in relation to one another while the cations remained constant. Thus two nutrient triangles, similar in some ways to those presented by SHIVE (8), were utilized. The advantage of this system was that the concentration of only three ions varied from square to square in any given triangle (fig. 1), while in SHIVE's system there

TABLE 1

EFFECT OF ADDITION OF SINGLE SALTS TO STANDARD THREE SALT NUTRIENT SOLUTION ON GROWTH OF BILOXI SOYBEANS (PER 100 PLANTS)

COMPOSITION OF NUTRIENT SOLUTION	TOTAL DRY WEIGHT (GM.)	DRY WEIGHT OF TOPS	DRY WEIGHT OF ROOTS	TOP/ROOT RATIO
A. Complete solution ($MgSO_4$ 0.0045 mol; $Ca(NO_3)_2$ 0.0060 mol; KH_2PO_4 0.0045 mol)	101.97	80.57	21.4	3.76
B. One-fifth complete; same as A but at one-fifth concentration	77.6	47.7	30.0	1.59
C. Same as B with 0.0108 mol $CaCl_2$ added	66.66	47.57	19.09	2.49
D. Same as B with 0.0216 mol $NaNO_3$ added	64.69	48.53	16.16	3.00
E. Same as B with 0.0081 mol $MgCl_2$ added	65.55	44.72	20.83	2.14
F. Same as B with 0.0081 mol Na_2SO_4 added	72.06	47.06	25.00	1.88
G. Same as B with 0.0081 mol KCl added	82.88	56.76	26.17	2.16
H. Same as B with 0.0081 mol NaH_2PO_4 added	48.33	33.33	15.00	2.22
I. Same as B with 0.0216 mol NaCl added	53.99	36.28	17.71	2.05
J. Same as A but at one-tenth concentration	67.97	40.27	27.77	1.45

is variation in six ions, a variation in a cation always being accompanied by an equal variation in a corresponding anion. The accomplishment of this involved the use of two triangle systems.

In one triangle (fig. 1) a series of nutrient solutions in which the anions varied were made up using three stock solutions. Solution A consisted of 0.0045 mol KNO_3, 0.0045 mol $Mg(NO_3)_2$, and 0.0060 mol $Ca(NO_3)_2$. Solution B consisted of 0.0045 mol K_2SO_4, 0.0060 mol $CaSO_4$, and 0.0045 mol $MgSO_4$. Solution C consisted of 0.0060 mol $Ca(H_2PO_4)_2$, 0.0045 mol $MgHPO_4$, and 0.0045 mol KH_2PO_4. Plants

at one apex received solution A; at another apex, solution B; and at the third apex, solution C. The solutions in the body of the triangle were made by mixing various proportions of solutions A, B, and C, as indicated in figure 1. The concentrations of cations in the three

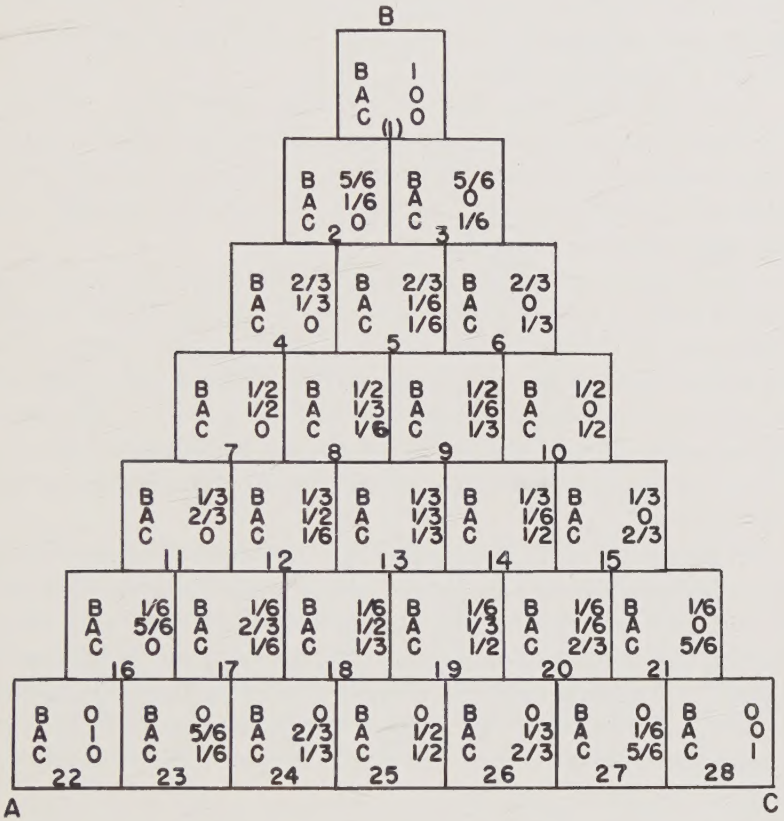

FIG. 1.—Triangle showing parts of solutions A, B, and C used in the twenty-eight different combinations. For example, square 13 contains one-third part solution B, one-third part solution A, and one-third part solution C.

solutions are practically constant, therefore these concentrations did not vary appreciably as the result of any mixture of the three solutions. In a similar manner a series of nutrients were made up in another triangle system, but in this case the cations varied and the anions remained constant. Solution D consisted of 0.0045 mol

$Mg(NO_3)_2$, 0.0045 mol $MgSO_4$, and 0.0045 mol $MgHPO_4$. Solution E consisted of 0.0045 mol KNO_3, 0.0045 mol K_2SO_4, and 0.0045 mol KH_2PO_4. Solution F consisted of 0.0060 mol $Ca(NO_3)_2$, 0.0060 mol $CaSO_4$, and 0.0060 mol $Ca(H_2PO_4)_2$. These solutions were utilized and mixed in a manner similar to that described for the first triangle, except that solutions D, E, and F were used in place of solutions A, B, and C respectively (fig. 1).

Biloxi soybeans were used as the final experimental material. The seeds were selected for uniformity, transplanted after germination, and watered, all as in the previous experiments. The pots were placed in the greenhouse at 75°–80° F. The relative humidity was generally above 60 per cent.

Three plants were placed in an 8×4-inch glazed jar containing quartz sand. Each set in the twenty-eight squares of the triangle contained ten jars for a total of thirty plants to each square. The sand was flushed with the various nutrient solutions daily and twice a week the sand was also flushed with distilled water. The plants in experiment I were planted October 1 and harvested October 21. The plants in experiment II were planted October 7 and harvested October 26.

At harvest the roots were washed free from sand and dipped into a saturated solution of sodium chloride to remove any particles adhering, then again washed and the plant divided into two fractions, (*a*) the tops, and (*b*) the roots and hypocotyls. Fresh weights were taken and the fractions were placed in a well ventilated drying oven at 80° C. for two days. Dry weights were then taken by means of a torsion balance sensitive to 0.01 gm. The data were recorded on a basis of 100 plants (figs. 2, 3).

Results

Experiment I

In this experiment the cations were constant throughout the series of solutions and the concentration of anions varied. Plants growing in the solution containing PO_4 as the only anion present (fig. 1, square 28) showed poor growth and evidences that the solution was toxic. The seedlings elongated at about the same rate as those in the other solutions, the cotyledons and first foliage leaves expanding

comparably. Soon after expanding, however, the primary leaves turned brown, and while still expanding the first two trifoliate leaves also developed discolored areas. The color change was from green to dark greenish brown, to light brown, to tan. The change was evident

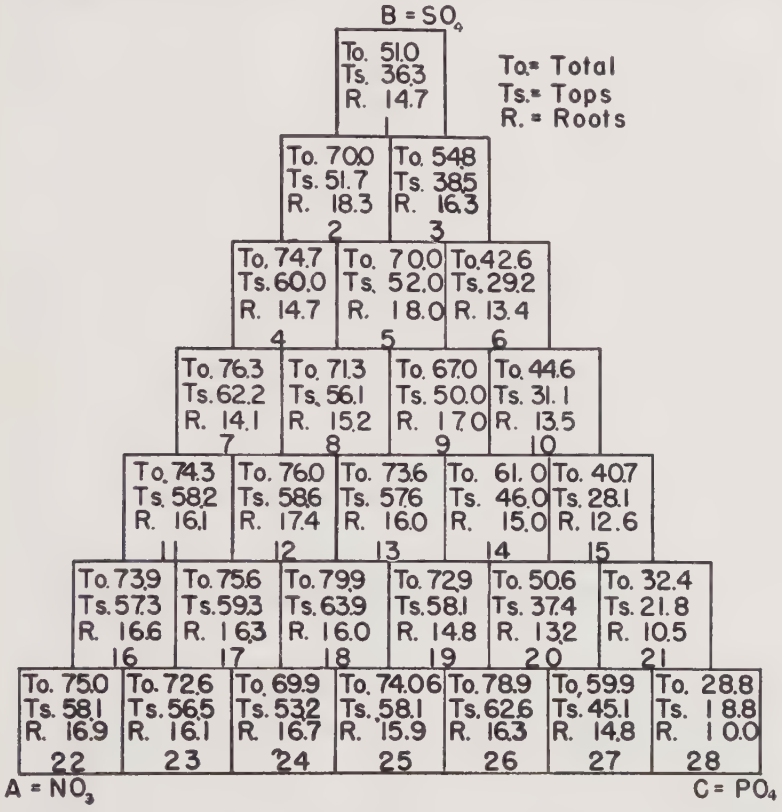

FIG. 2.—Dry weight accumulation per 100 plants of twenty-eight different solutions in which the cations remained constant and the anions varied.

first along the midribs and veins and then throughout the leaf, spreading from the veins. All the plants supplied with nutrient solutions deficient in nitrogen and containing phosphorus showed some evidences of these symptoms, and their amount and severity were in proportion to the amount of phosphorus present in the nutrient medium. Apparently these symptoms represent evidences of phosphorus toxicity. If small amounts of nitrates were present in the

nutrient solution, phosphorus toxicity was apparent only in those solutions containing comparatively high amounts of phosphorus. Thus no evidences of phosphorus toxicity were apparent in those solutions (fig. 1, squares 5, 13, 25) in which the values of both NO_3

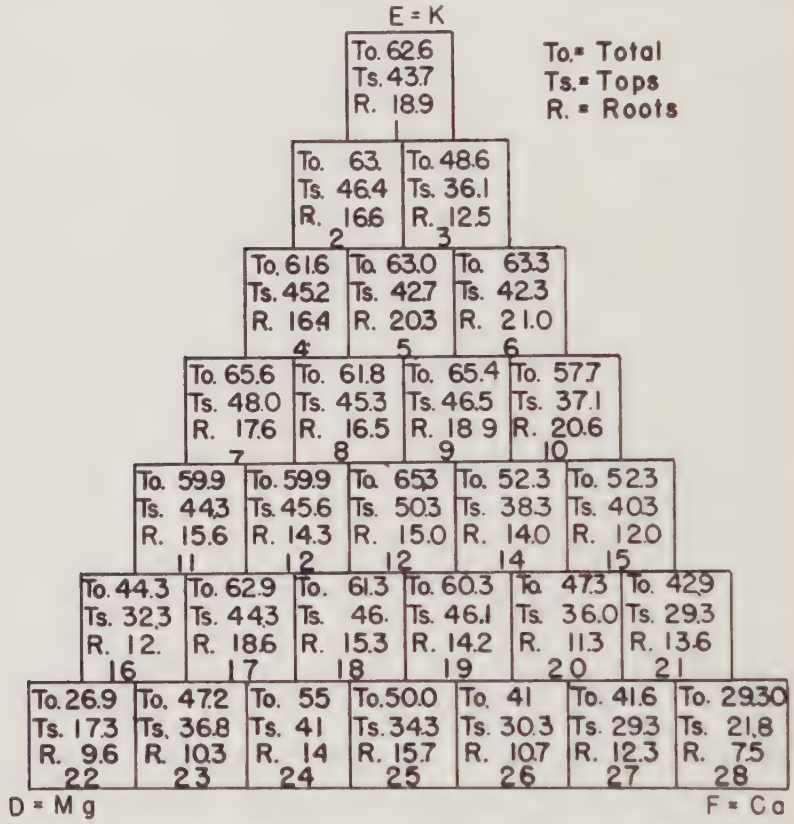

FIG. 3.—Dry weight accumulation per 100 plants of twenty-eight different solutions in which the anions remained constant and the cations varied.

and PO_4 were at one-sixth, one-third, or one-half their maximum. But in the solution in which the NO_3 was at one third its maximum value and the PO_4 at two-thirds its maximum value, toxicity symptoms were apparent during early stages of growth (fig. 1, square 26). As the plants grew older these symptoms did not increase in severity and new growth seemed to be but slightly affected. In the solution containing one-sixth the maximum amount of NO_3 and one-third the

maximum amount of PO_4 (fig. 1, square 9) the symptoms developed much the same as just described, except that new growth continued to display a few discolored areas even up to the time of harvest. Thus there is a relationship between the relative concentration of nitrate and phosphate in the nutrient and the development of phosphate toxicity symptoms, a small amount of nitrate seemingly serving in some measure to counteract what otherwise might be a toxic concentration of phosphorus. The relationship between various concentrations of phosphate and nitrate ion in the accumulation of dry weight by the plants is shown in figure 2. In general, at any particular concentration of phosphorus, more dry weight was produced with increasing concentrations of nitrate.

When nitrate only was the major anion present (fig. 1, square 22), a rate and type of growth took place which compared favorably with the greatest growth in any of the other solutions, although the total dry weight was slightly lower than in a few of the other solutions (fig. 2, squares 7, 12, 18, 26). In no case did distinct symptoms of phosphorus or sulphur deficiency appear. This may have been because during germination and early growth, such as is represented by the period of these experiments, adequate supplies of these elements were available from storage supplies in the seed. It is also possible that, even though these elements were not in the nutrient solutions supplied to certain plants, these plants may have obtained some of them from the traces present in the sand but which was free from nitrogen and phosphorus. (The seedlings were germinated in sand and watered with tap water during the early stages of germination before transplanting.) There were no evidences of sulphate toxicity or deficiency at any time.

As the amount of PO_4 increased in the solution the importance of NO_3 for vegetative growth and accumulation of dry weight became much more evident (fig. 2). PO_4 may constitute as much as two-thirds the concentration of the anions present and yet active growth result if NO_3 is present as a third part (fig. 2, square 26).

Experiment II

Throughout the series in this experiment the anions were present at nearly the same concentration and the cations varied in relation to one another. At the time of harvest the plants showed a great

many differences between the various lots. Certain distinct characteristics varied in intensity from lot to lot. Thus some showed characteristic reddening of the petioles and midribs of the leaves, others reddish brown spots in the blades. While both these symptoms were apparent in some lots, others showed one and not the other. Because of this, certain symptoms which were characteristic of more than one lot were selected and their presence or absence recorded for each group. These patterns of response or symptoms are as follows, with the symbols used to designate them.

o Presence of red coloration:
  - o In petiole of primary leaves
  - oo In petiole of primary leaves and upper leaves
  - ooo In petiole and midrib of primary leaves and upper leaves
  - oooo In petiole and midrib of primary leaves and upper leaves and in the stem

\# Presence of reddish brown spots in blade of leaf (no relative degrees of this were noted; it was either present or absent)

= Presence of light brown areas in blade of leaf; these differed from the preceding in that the color was lighter and more widespread in the leaves

g Chlorosis:
  - g Partial chlorosis of some leaves
  - gg Chlorosis general
  - ggg Chlorosis more general
  - gggg Chlorosis extreme

The presence of these symptoms and the degree to which they were manifested are shown in figure 4. A comparison of the results indicates that the reddish coloration of petioles and midribs (symptom o) represents an effect of Ca toxicity, since it is present in all plants which receive relatively high concentrations of Ca, regardless of the relative amounts of the other ions. The reddish brown spots in the blades (symptom #) represent evidence of Mg deficiency, since the symptom is present in all plants growing in magnesium-deficient solutions. The presence of light brown areas in the leaves (symptom =) represents evidence of calcium deficiency, since it is shown by all plants which grew in solutions deficient in calcium. It

is more difficult to interpret the chlorotic effects, but the presence of a high concentration of potassium seems to inhibit development of chlorosis, as does the presence of all three elements in a balanced condition. As for the growth response, the greatest correlation be-

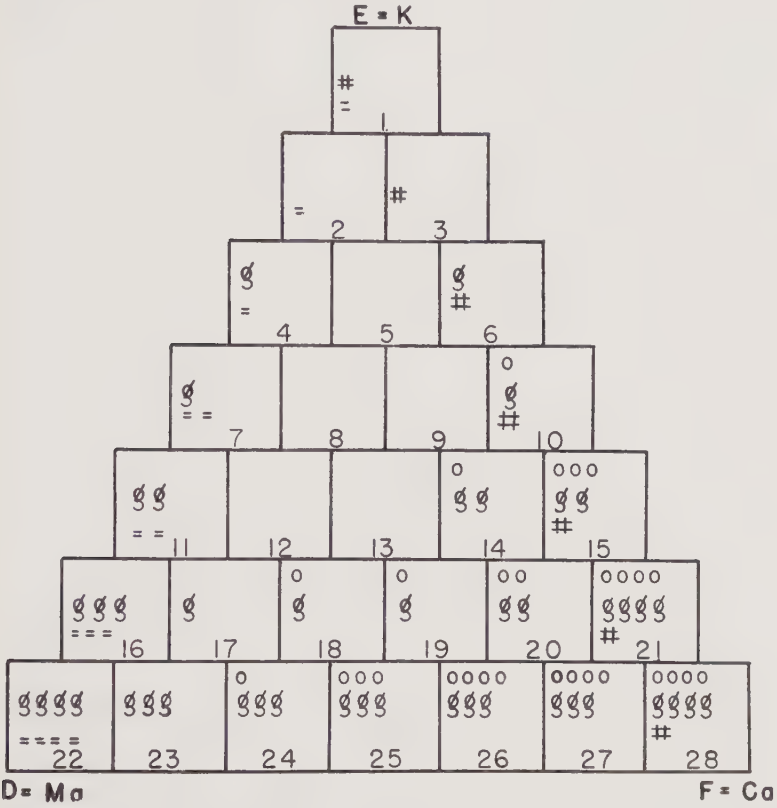

FIG. 4.—Toxicity and deficiency symptoms in twenty-eight different solutions in which the anions remained constant and the cations varied.

tween growth and variation in some ion is found in the case of variations in potassium. Thus high concentrations of potassium favor active vegetative growth even though both calcium and magnesium are very low in concentration in the solution (fig. 3).

On the basis of chemical analysis of plants, several investigators have shown that there exists a fairly definite relation between their phosphorus and nitrogen contents and the type of growth or stage

of development at the time of taking samples for analysis. The present investigation has further demonstrated that such a relation between nitrogen and phosphorus in the nutrient solution also affects the rate of growth of the plants and the appearance or lack of appearance of certain toxic symptoms. LEMMERMAN and BEHRENS (6) obtained satisfactory growth of oats in pots when the proportion between P_2O_5 and nitrogen was 1:1 but not when it was 3:1, in which case there was phosphate poisoning. DANIEL (3) found the nitrogen and phosphorus ratios within the plant tissue closely correlated. HUTCHINGS (5) found a close relationship in soybean between the phosphorus and nitrogen content of the plant material. BREAZEALE (1) stated that the absorption of nitrogen by wheat seedlings was not affected by the presence of either phosphorus or potassium; absorption of phosphorus was slightly increased by the presence of nitrogen. MOORE (7) found that the phosphate ion ordinarily used in nutrient solutions was injurious to peanut seedlings when grown in the light, but those grown in darkness were not subject to phosphate toxicity.

The relationship between phosphorus and nitrogen in soybean is important in overcoming toxicity of phosphorus. Phosphorus is toxic in very small concentrations if nitrogen is lacking, but it is not toxic even in fairly high concentrations if larger amounts of nitrogen are present. In general, one part of NO_3 will overcome the toxic effects of two parts of PO_4. In older plants this ratio may not be the same, seedlings being more susceptible to phosphate poisoning than older plants. This is possibly caused by the greater absorption of phosphorus at the seedling stage, the lower tolerance for phosphates, or the amount of phosphorus stored in the seeds. It is possible that the older plants have enough stored nitrogen to tolerate an outside ratio which under other circumstances would be toxic.

The writer wishes to express his appreciation of the helpful suggestions given by Dr. K. C. HAMNER during the course of these experiments.

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